THE ULTRASTRUCTURE OF THE AESTHETES IN *LEPIDOPLEURUS CAJETANUS* (POLYPLACOPHORA: LEPIDOPLEURINA)

FRANZ PETER FISCHER
INSTITUT FÜR ZOOLOGIE, TECHNISCHE UNIVERSITÄT MÜNCHEN
LICHTENBERGSTRASSE 4, D 8046 GARCHING,
FEDERAL REPUBLIC OF GERMANY

ABSTRACT

The aesthetes of *Lepidopleurus cajetanus* Poli consist of five different cell types: one or two photoreceptor cells are present in the periphery in many of these organs. Products of tall secretory cells pass through a perforated apical cap to the outside. Central cells probably are chemoreceptors. Micraesthete cells form lateral branches from the main stem and end with unperforated caps at the shell surface; their function is unknown. Peripheral cells form most of the border to the calcareous shell substance. It is proposed that this is the general composition of the aesthetes in chitons.

Aesthetes are numerous organs in the upper shell layer of the Polyplacophora (Figs. 1, 2). In recent years their fine structure has been studied in several species. Except for the species *Acanthochitona fascicularis* L. (Acanthochitonina) (Fischer, 1979), only members of the Chitonina have so far been examined in this respect (Boyle, 1974; Haas and Kriesten, 1978; Fischer and Renner, 1978; Baxter *et al.*, 1987). For the discussion on the function of these unique organs it is important to know which features are constant in the aesthetes and which are species-specific variations. In the present paper the aesthetes of a member of the relatively primitive suborder Lepidopleurina are described and their possible functions are discussed.

MATERIAL AND METHODS

Adult polyplacophorans of the species *Lepidopleurus cajetanus* Poli were collected in the subtidal (about 1 m below low tide level) region on the coast of northern Yugoslavia. Parts of the tegmental shell layer containing the aesthetes were removed and fixed in 5% glutaraldehyde in phosphate buffer (pH 7.4) for two hours and postfixed in 2% osmium tetroxide for two hours, all at 3ºC. After dehydration in ethanol and propylene oxide the specimens were embedded in Durcupan. Some of the specimens were decalcified overnight in chilled 3% EDTA in phosphate buffer after glutaraldehyde fixation. The others were split into two pieces and the calcareous parts were removed by use of 5% HCl after embedding. Since the tissue is already penetrated by the embedding material, no damage occurred to the cells during this procedure, following which the specimens again were embedded in Durcupan to fill the holes left by the calcareous parts. Ultrathin sections were cut with a LKB or a Reichert ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds, 1963) and studied in a Zeiss or a Jeol electron microscope.

For scanning electron microscopy, the organic material in the shell valves was removed by the use of concentrated KOH at room temperature for about one hour, cleaned in an ultrasonic cleaner and air dried. Other shells were air dried without previous treatment. The specimens were given a 300 Å thick coating with gold and were examined in a Cambridge SEM.

RESULTS

SHELL SURFACE

The head valve has the shape of a half circle with a few concentric ribs on the surface. In contrast, the other seven valves show two different surface areas (Fig. 3): the lateral fields resemble the head valve; parallel ribs are oriented along the long axis of the animal in the second to the seventh valve and are semicircular in the last one. In the median area of the valves II-VIII, 60 μm-wide elevations form rows that run mainly in the long axis. Parts of the articulamentum, the apophyses, protrude anteriorly to form a joint with the valve in front.

On the top of the elevations, as well as on the ribs, the openings of the aesthetes can be seen (Fig. 4), with a megapore (diameter= 11-14 μm) in the center, surrounded by 4-9 micropores (diameter= 9 μm). On the lateral areas, there are more micropores per megapore than in the median fields. The same is true for the absolute number of the aesthetes

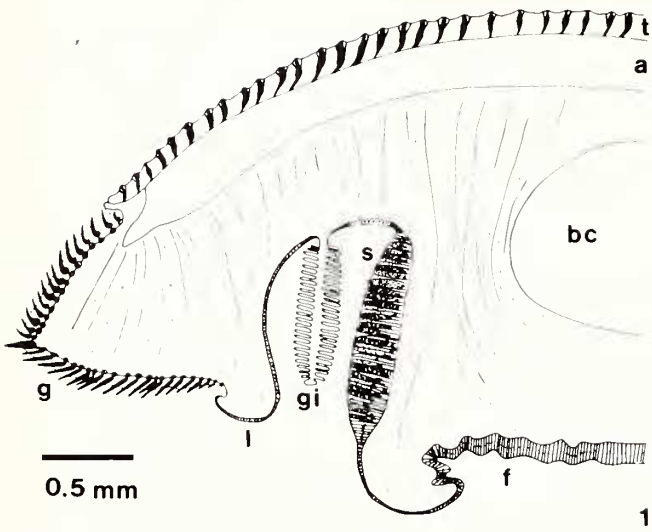

Fig. 1. Schematic cross section through an adult *Lepidopleurus cajetanus,* left half (a, articulamentum; bc, body cavity; f, foot; g, girdle covered with spicules; gi, gill; l, lateral fold; s, secretory cells of the foot epithelium in the pallial groove; t, tegmentum with numerous incorporated aesthetes) (adapted from Maile, 1981).

(number of megapores), with about 150 per mm² in the lateral and 90 per mm² in the median area. The head valve has the highest density of aesthetes, about 200 per mm².

In untreated shell valves, each megapore is filled with the apical cap of the main stem (= megalaesthete) of an aesthete (Fig. 2). Each micropore contains the subsidiary cap of a micraesthete, which is a branch from the megalaesthete. In older aesthetes, the apical caps show a perforation with many pores of about 0.1 μm in diameter (Fig. 5). The subsidiary caps do not exhibit such a pattern. In young aesthetes the apical cap is completely covered by the periostracum.

AESTHETES

The aesthetes are, like the papillae in the girdle, extensions of the epidermis. Most of the cells of the aesthete are still connected with the epithelium via the aesthete canal. Some of these basal cell extensions are nervous elements and run further to the lateral nerve cords.

Each aesthete is about 110 μm long and 30 μm thick. It contains 35 to 40 cells of five distinct cell types: secretory cells, central cells, photoreceptor cells, micraesthete cells branching from the main stem, and peripheral cells (Fig. 2). Except for the micraesthetes, every type can exhibit a basal extension to the epithelium (Fischer, 1978a); for the micraesthete cells the situation is not yet clear. At the shell surface the main stem is covered by the apical cap and each micraesthete by a subsidiary cap.

APICAL CAP. The apical cap consists only of organic material and can be divided into two zones (Fig. 2): the distal part, containing numerous parallel pores and the proximal part, consisting of a network of thin filaments of two types. There are filaments of about 80 nm in diameter, which form

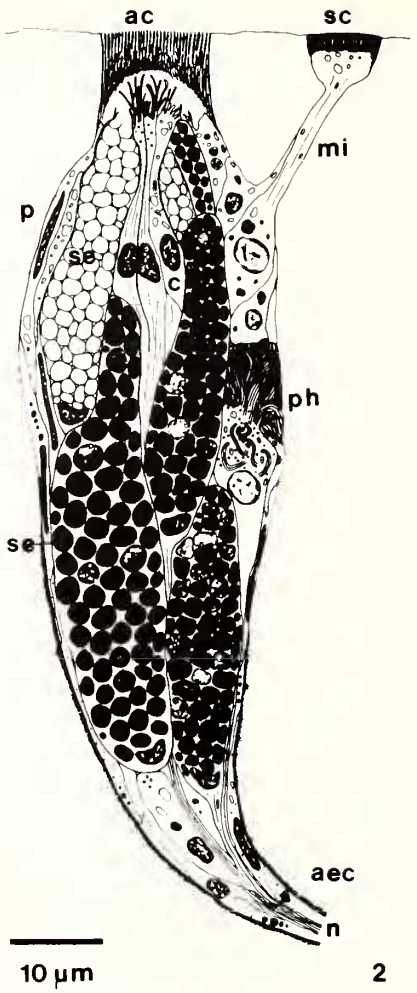

Fig. 2. Schematic longitudinal section through an aesthete (ac, apical cap; aec, aesthete canal; c, central cell; mi, micraesthete; n, neurites; p, peripheral cell; ph, photoreceptor cell; sc, subsidiary cap; se, secretory cell).

the skeleton, from which 25 nm wide filaments branch off (Fig. 6). These fine filaments form the border of the cap to the interior of the aesthete.

SECRETORY CELLS. Each aesthete has three to eight tall secretory cells of different forms. Some of them, especially in young aesthetes, show a high metabolic activity in the proximal part; granular endoplasmic reticulum (ER), a few Golgi apparatus and numerous mitochondria surround an active nucleus. The secretory granules produced are stored distally. Most of the secretory cells are densely filled with membrane bound secretion granules of various electron densities (Fig. 7). The nucleus lies basally, its chromatin is highly condensed (Fig. 8). Remains of endoplasmic reticulum and a few mitochrondria are often present nearby. One or two secretory cells that open distally secrete material beneath the apical cap. Some cytoplasm between the former granules remains; the interior is now continuous with the extracellular space beneath the apical cap (Fig. 9). In the neighbourhood of these

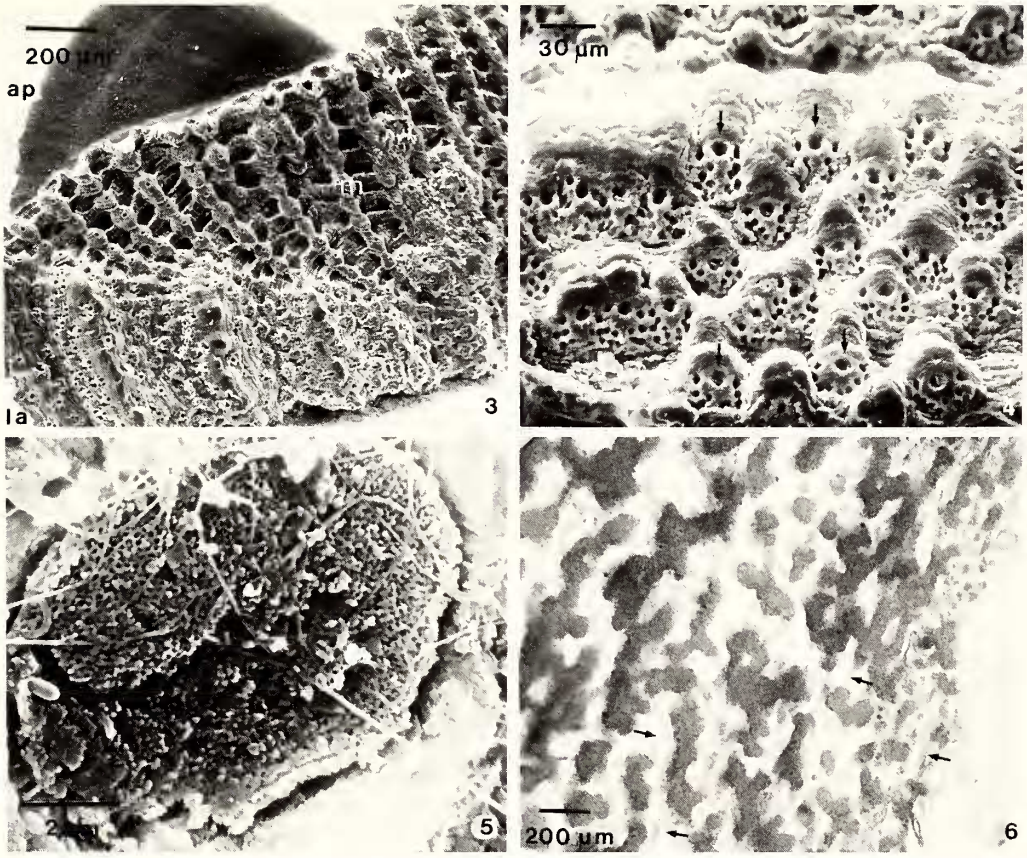

Fig. 3. Left half of an intermediate shell valve. KOH-treated (ap, apophyse; la, lateral triangle; m, median triangle). **Fig. 4.** Higher magnification of the lateral triangle, KOH-treated (arrows indicate several smaller micropores surrounding a megapore). **Fig. 5.** Surface of an apical cap. The cap is perforated by numerous small pores. **Fig. 6.** Longitudinal section of the basal area of an apical cap consisting of a network of larger and smaller (arrows) organic filaments.

cells, some of the peripheral cells exhibit characteristics of decomposing cytoplasm; lysosomes and autophagous vacuoles surround an active nucleus.

CENTRAL CELLS. The central cells ("sensory cells" according to Boyle, 1974) (as the photoreceptor cell is also sensory, I use the more neutral term "central cell") of *Lepidopleurus cajetanus* are prominent compared with the other species studied so far. The nuclei of all central cells (about five per aesthete) are situated in the distal part of the aesthete. Distally, underneath the apical cap, each central cell forms numerous microvilli and one cilium (9+2 structure) (Fig. 10). The cytoplasm of the central cells contains numerous mitochondria and microtubules running along the long axis of the cells. Distally the cytoplasm is filled with clear 0.3 µm wide vesicles. The central cells are connected together by zonulae adhaerens and septate junctions.

PHOTORECEPTOR CELLS. Most of the aesthetes (but not all of them, irrespective of the position in different valve areas) contain one or two photoreceptor cells. They lie peripherally in the aesthete and do not exhibit a special orientation pattern, such as being always located on the same side of the aesthete body, as it is the case in *Chiton olivaceus* Spengler (Fischer and Renner, 1978). As in other species, they show two distinct areas, the cell body and the rhabdomere (Fig. 11). The microvilli (0.05-0.1 µm in diameter) of the rhabdomere branch from the whole distal part of the cell; they have no regular orientation. Their cytoplasm contains small granules. One or two cilia (9+2 structure) can be present.

The nucleus is relatively large (6.5 µm) and has only a little condensed chromatin. In the perikaryon, numerous mitochondria, microtubules, glycogen and multivesicular bodies are present. A specialized agranular ER forms large areas of parallel membrane cisternae that are connected with the granular ER. Laterally these cisternae give off numerous clear vesicles (40-170 nm) that are found up to the rhabdome.

MICRAESTHETES. All micraesthetes branch off from the same zone of the main stem. Their nuclei lie in this area; they are large (6 µm) and have only little condensed chromatin. Here and in the "arm" (the part between the main stem of the aesthete and the tip of the micraesthete cell) we find numerous mitochondria and microtubules along the long axis (Fig. 12). In the basal part, multivesicular bodies or lysosomes are frequently found. Peripheral cells surround the

proximal part of the "arm"; both cells show invaginations into the other cells. The "head" (the tip of the micraesthete cell) is slightly swollen and also contains mitochondria. The distal part forms numerous microvilli towards the subsidiary cap (Fig. 13). The "head" can show a high degree of vacuolization in some micraesthetes, but this zone does not continue into the "arm".

SUBSIDIARY CAP. In contrast to the apical cap, the subsidiary caps appear continuous at their outer and inner surfaces. They also consist of organic material. They contain inner pores (width= 0.1 μm), but in nearly all cases they are closed to the outside, as well as to the interior of the micraesthete, by continuous sheets. The distal sheet is up to 0.2 μm, the proximal sheet about 0.1 μm wide.

In some cases the micraesthete cap has been damaged by organisms. In these cases, parallel sheets of varying thickness are placed underneath the remainder of the cap. Sometimes, the subsidiary cap is completely replaced by this structure.

PERIPHERAL CELLS. The peripheral cells surround the

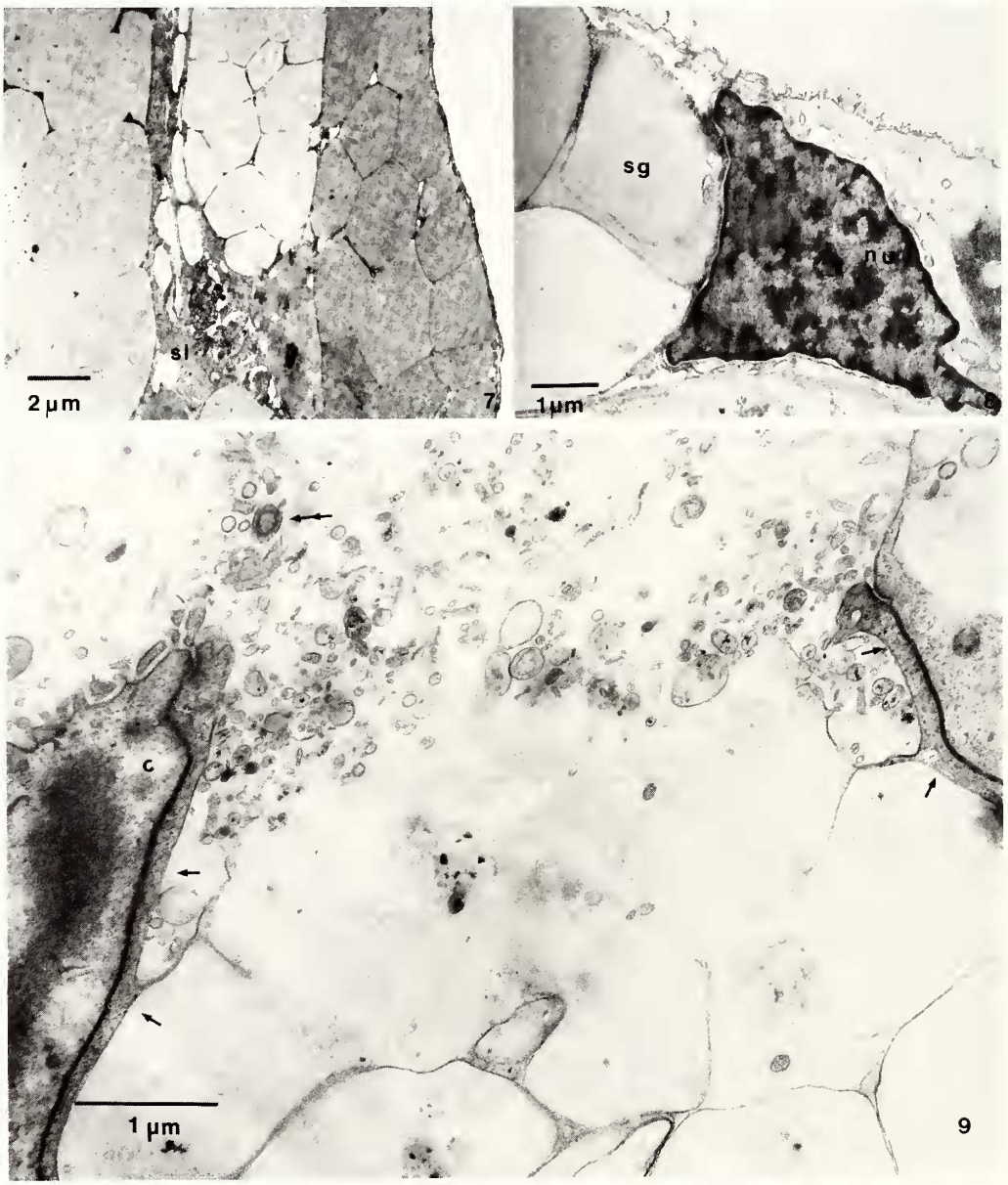

Fig. 7. Longitudinal section of an aesthete nearly completely filled with the granules of secretory cells (sl, secondary lysosome). **Fig. 8.** Base of a secretory cell (nu, highly condensed nucleus; sg, secretion granule). **Fig. 9.** Distal tip of a secretory cell after secretion. Surrounding cytoplasm (arrows) and small cytoplasmic remains are visible between the former secretion granules. The interior is now continuous with the extracellular material underneath the apical cap, in which microvilli and cilia (double arrow) of central cells are embedded (c, central cell).

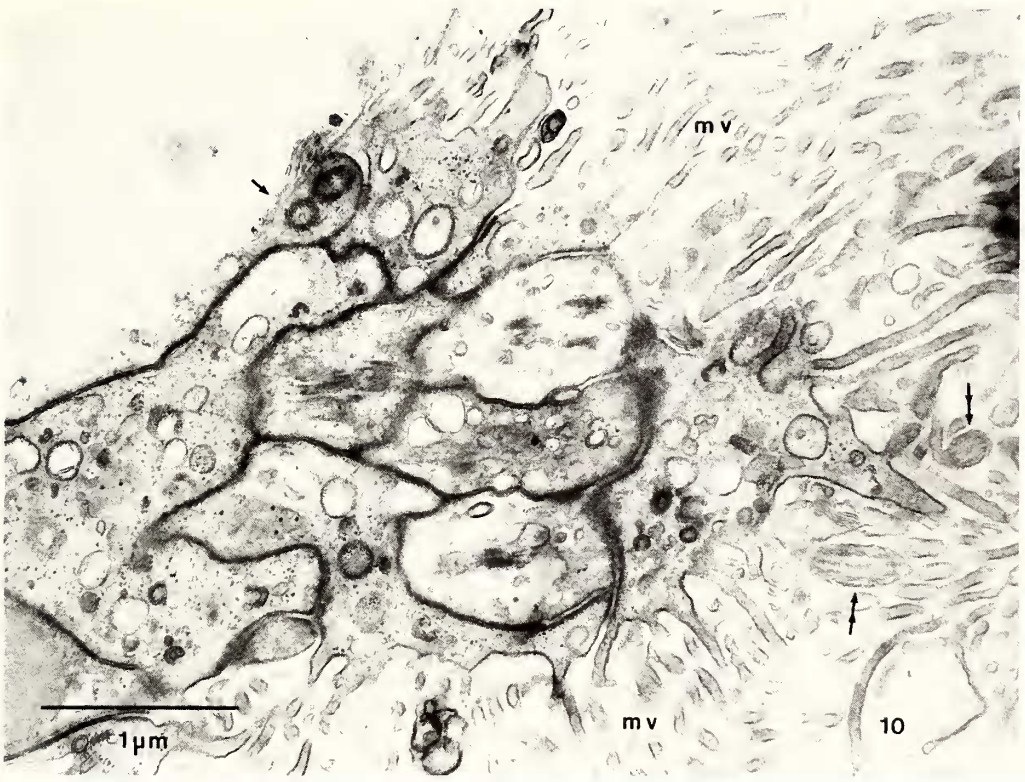

Fig. 10. Distal tip of central cells with protruding microvilli (mv) and cilia (double arrow). Arrow indicates basal body in a central cell.

body of the aesthete as a sheet about 0.75 µm in width. They are not present in all parts of an aesthete (e.g. Fig. 7). The fine structure varies considerably, e.g. in the content of decomposing structures. In the basal part of the aesthete, peripheral cells form an extracellular sheet of fine filaments into the shell material. Some of the filaments protrude, roughly perpendicularly, far into the shell substance.

AESTHETE CANAL. The aesthete canal is surrounded by peripheral cells (Fig. 14). In the center, various fibers (basal extensions of the aesthete cells to the epithelium) run towards the epithelium under or lateral to the shell valves. Some of the fibers connect the secretory cells with the epithelium; these fibers contain mitochondria and microtubules. About ten of the fibers are much thinner than those of the secretory cells (0.4 versus 1.5 µm). They are densely filled with microtubules and are probably nervous elements (Fig. 14). Structures resembling neurosecretory elements are also a regular feature in this area.

DISCUSSION

The general structure of the aesthetes of *Lepidopleurus cajetanus* is very similar to that of previously studied species. Despite the differences in the architecture of the shell valves, there is no major difference between the aesthetes in all three extant polyplacophoran suborders. The aesthetes are obviously an evolutionarily old system in the chitons.

The different cell types, each with pronounced ultrastructural characteristics, suggest that the aesthetes are compound organs with both a sensory and a secretory function. It is not clear whether some or all cell types work together to perform a more complex function or whether they function more or less independently.

The secretory cells produce secretions basally and release them apically. In *Chiton olivaceus*, animals outside the water show an increased secretory activity (Fischer, 1978a). Additionally, recordings with a glass microelectrode show slow rhythmic changes in the electrical potential under the same conditions (Fischer, unpub. data). This could suggest that one function of the secretion is to prevent the desiccation of the aesthetes during low tide. However, species that prefer to live in deeper water also have well developed secretory cells (Baxter *et al.*, 1987). The secretions probably have other protective functions, e.g. against predators or organisms growing on the shell. One indication for such a function is that the pores of the apical cap open only in older aesthetes, whereas newly formed aesthetes are covered by the periostracum.

The role of the central cells is not known. In their fine structure they resemble chemoreceptors of insects (Ernst, 1969). The position of this cell type underneath the perforated apical cap, the pronounced membrane proliferations (microvilli and cilia), and the high metabolic activity support such a hypothesis. Structures resembling nervous elements were

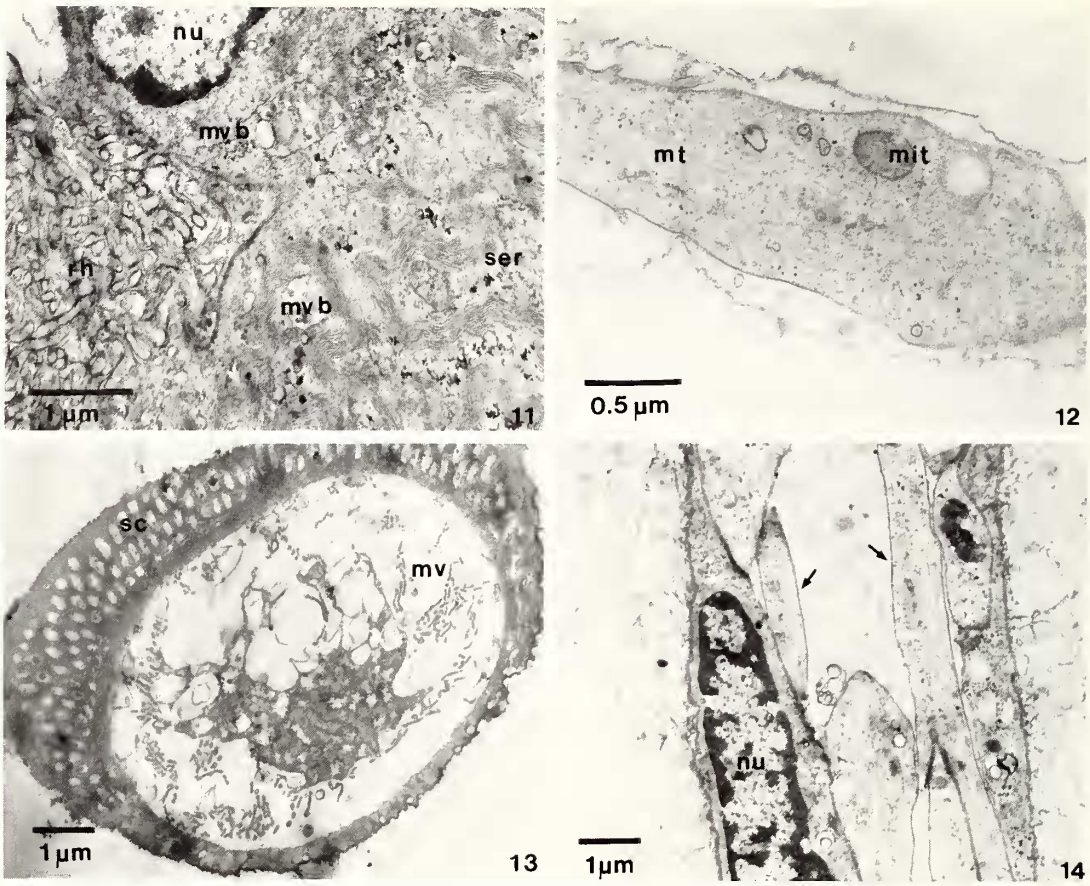

Fig. 11. Photoreceptor cell at the transition of perikaryon and rhabdomere (mvb, multivesicular body; nu, nucleus; rh, rhabdomere; ser, specialised agranular ER). **Fig. 12.** Longitudinal section of the "arm" part of a micraesthete cell (mit, mitochondrion; mt, microtubules). **Fig. 13.** Subsidiary cap (sc) with the microvilli (mv) of the underlying micraesthete cell. **Fig. 14.** Longitudinal section through an aesthete canal (nu, nucleus of a peripheral cell). Arrows indicate profiles resembling neurites.

found near the base, but their relationship to the central cells is not clear. A possible function could be to detect desiccation and/or animals grazing on the shell (and eating also the distal parts of the aesthetes), with a subsequent reaction of the secretory cells. *Lepidopleurus cajetanus* has the best developed central cells of all species studied so far; in the Lepidopleurina the articulamentum is lacking in broad shell areas and the aesthetes are connected directly with the dorsal epithelium. Destruction of the aesthetes and a subsequent invasion of microorganisms into empty aesthete canals could be much more severe in this group than in the Ischnochitonina or the Acanthochitonina.

The photoreceptor cells resemble in detail the photoreceptor cells of *Chiton olivaceus* and *Acanthochitona fascicularis* (Fischer, 1978b, 1979; Fischer and Renner, 1978) as well as the photoreceptor cells in the two different shell-eye types in the chitons (Boyle, 1969; Haas and Kriesten, 1978). Boyle (1974) found no typical photoreceptor cells in the aesthetes of *Lepidochitona cinerea* L., but he described "microvillous areas" and areas with "lamellate bodies". These very likely correspond to the rhabdomere and the agranular ER of the photoreceptor cells. This cell type is the only one in the aesthete that shows ultrastructural differences between light- and dark-adapted animals (Fischer, 1978a). Additionally, experiments with partially masked *C. olivaceus* clearly show that the shell valves contain photoreceptive elements (Fischer, unpub. data). As a common feature, photoreceptor cells seem to be a primary part in chiton aesthetes.

At first sight, it is astonishing that the shell valves contain so many, and simple, photoreceptive elements. In some species, aesthetes in certain shell areas have been transformed into eyes of various complexity (Boyle, 1969; Fischer, 1978a; Haas and Kriesten, 1978). Most species, however, have only the "normal" aesthete type. The situation could be comparable with other invertebrates, like the earthworm which avoid light during the day and feed at dawn and when it is dark. The earthworm also has many primitive light receptors dispersed in the skin. As behavioural studies show (Fischer, unpub. data), the photoreceptor cells in the aesthetes have a similar function. Most chiton species avoid bright sunlight and hide below stones or in the mud during the day. Chitons with masked shells do not exhibit such a behaviour (except species that also have photoreceptor cells in their girdle

papillae, e.g. *Acanthochitona fascicularis*). Chitons that live in deeper water obviously have lost their photoreceptor cells. Baxter *et al.* (1987) found no photoreceptor cells in the aesthetes of *Tonicella marmorea* Fabricius.

The function of the micraesthetes is still completely obscure. Their high density in the shell valves suggests that they play an important role in the biology of the Polyplacophora. Among all species studied, only some of the lateral aesthetes in *Acanthochitona fascicularis* lack micraesthetes (Fischer, 1979). Baxter *et al.* (1987) showed that in *Tonicella marmorea*, the micraesthetes contain numerous lamellate granules. They suggest that micraesthetes and the megalaesthete produce periostracum material. This hypothesis has already been put forward by Nowikoff (1907). In all the species I studied, no secretory granules in the micraesthetes could be found. In addition, the subsidiary cap does not allow a penetration of material from the inside of the aesthete to the outside. In electrical recordings from the area underneath the subsidiary cap in *Chiton olivaceus*, no indication of a secretory process could be found under many different experimental conditions (Fischer, unpub. data). In other species, the subsidiary cap looks similar to that of *Lepidopleurus cajetanus* (Fischer and Renner, 1978; Haas and Kriesten, 1978) or are much thinner but without inner pores (Boyle, 1974). Both types can be present in different shell areas in certain species (Fischer, 1979). Baxter *et al.* (1987) showed that in *T. marmorea*, microvilli of the micraesthete cell protrude into the subsidiary cap. However, the distal surface of the cap also seems to be continuous in this species. In all species studied so far, the continuous part of the subsidiary cap is about 0.4 μm, irrespective to whether inner pores are present. Certainly there is a great need to study the aesthetes of species that differ in their ecology from the species studied so far, in order to gain a better understanding of the function of the micraesthetes.

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